CHAPTER 23

Adenoviruses

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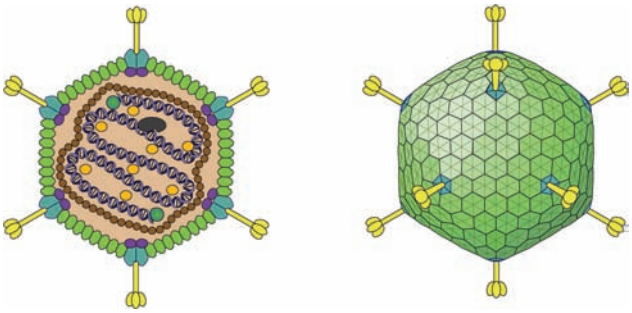

Adenoviridae

From Greek *adenos* (gland), for site of first isolation, adenoids

VIRION

Naked icosahedral capsid (T = 25) with 20 triangular faces.
Diameter 70–90 nm.
Knobbed fibers protruding from each of 12 vertices.
Eleven proteins in virion.

GENOME

Linear, double-stranded DNA, 30–36 kb.
Short, inverted terminal repeat sequence.
Both 5' ends covalently bound to a virus-coded terminal protein.

GENES AND PROTEINS

Codes for up to 50 proteins.
Most regions of both DNA strands are transcribed by cellular RNA polymerase II.
Six early and two delayed early transcription units: E1A, E1B, E2A, E2B, E3, E4; IVa2, IX.
One major late transcription unit: five subclasses of late mRNAs, L1 through L5.
Each transcription unit gives rise to multiple mRNAs by RNA splicing.
Two small VA RNAs transcribed by RNA polymerase III.

VIRUSES AND HOSTS

Fifty human serotypes in six subgroups (A through F).
Other adenoviruses in cattle, mice, birds, etc.

DISEASES

Respiratory syndromes including pneumonia, but not common colds.
Eye and gastrointestinal infections.

DISTINCTIVE CHARACTERISTICS

Multiple mRNAs arise by extensive alternative splicing.
Late transcripts are made from a single strong promoter and five alternative polyadenylation signals.
DNA synthesis is primed by terminal protein, uses viral DNA polymerase.
Can cause tumors in experimental animals but are not known to cause tumors in humans.
Adenoviruses are widely used as gene therapy and anticancer virus vectors.

Adenoviruses cause respiratory and enteric infections in humans

Adenoviruses were first isolated by Wallace Rowe in 1953 from tissue culture cell lines derived from tonsils and **adenoid** tissues of sick children. Shortly afterward, Maurice Hilleman and colleagues identified the same agent as the cause of a respiratory epidemic in army recruits in the United States. Originally (and incorrectly) postulated to be a cause of the common cold, adenoviruses were subsequently shown to be responsible for a number of other respiratory syndromes including pneumonia; they can also cause eye and gastrointestinal infections. The current adenovirus name and nomenclature were adopted in 1956, acknowledging the original source of the virus from adenoid tissue.

Adenoviruses can be oncogenic, but do not cause cancer in humans

Human adenovirus type 12 can induce malignant tumors in hamsters. This was the first human virus shown to be oncogenic. Although no evidence has linked adenoviruses to human cancer, these viruses have been used as models for understanding the molecular biology of cancer. Furthermore, their study has helped uncover basic mechanisms of gene expression in mammalian cells. In 1977, the laboratories of Philip Sharp and Richard

Roberts discovered RNA splicing by studying adenovirus late mRNAs, and were jointly awarded the Nobel prize for this discovery. In 1979, Robert Roeder used the adenovirus major late promoter to demonstrate specific initiation of transcription by RNA polymerase II *in vitro*, and he and others subsequently discovered many of the protein factors involved in transcription in mammalian cells by using this model system. Human adenoviruses have recently become of great importance as gene therapy vectors and are one of several promising new oncolytic virus anticancer agents.

The 100 known adenoviruses include 50 human virus serotypes, distinguished by their sensitivity to neutralizing antibodies. The human viruses are further classified into six subgroups (A to F) based on their ability to agglutinate red blood cells. This chapter will deal with the best-studied human adenoviruses: types 2 and 5, non-oncogenic members of group C, and type 12, a highly oncogenic member of group A.

Virions have icosahedral symmetry and are studded with knobbed fibers

Adenovirus virions have a remarkable resemblance to a regular icosahedron, with 20 equilateral triangular faces and 12 vertices (Figures 23.1 and 23.2). This handsome capsid contains a total of 11 viral proteins, but is constructed from two basic building blocks, the **hexon** and the **penton base** (Figure 23.2).

Hexons consist of trimers of the hexon protein (polypeptide II); each hexon has six nearest neighbors, hence the name. A total of 240 hexons cover the 20 faces

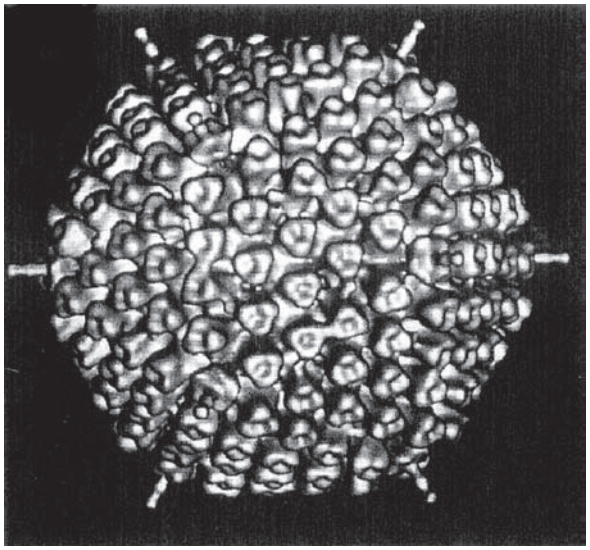

Figure 23.1 Adenovirus virion. A computer-generated image of adenovirus type 2 virion, viewed along a threefold symmetry axis. Reconstructed from cryoelectron microscopy images.

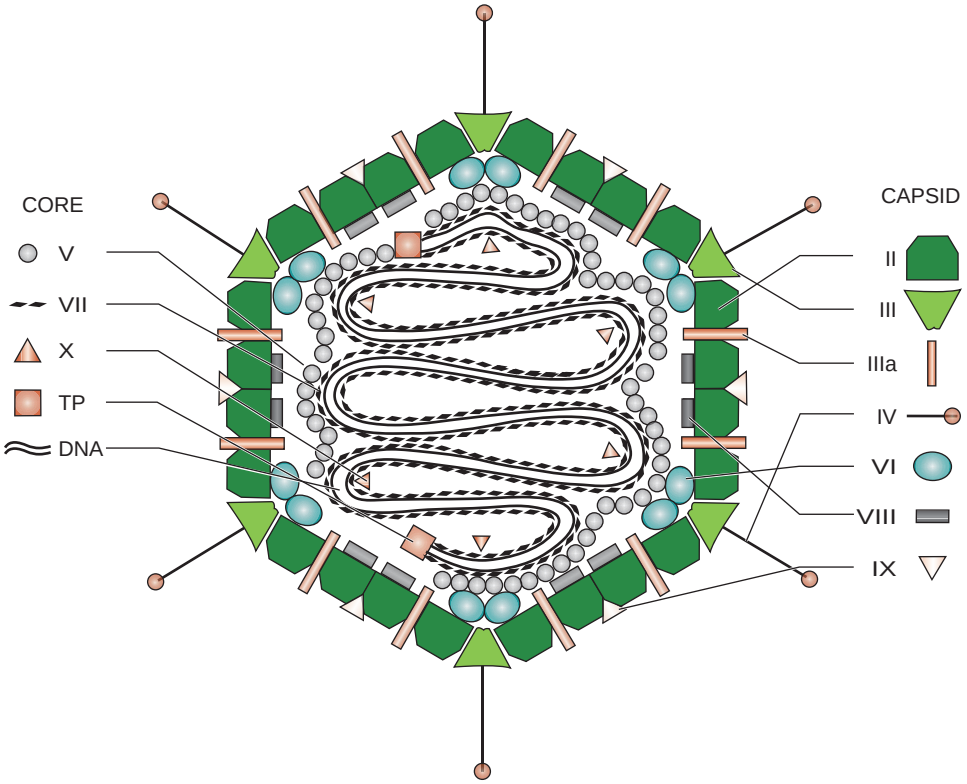

Figure 23.2 Structure of adenovirus virion. A schematic cross-sectional view of a human adenovirus type 5 virion, with components shown as indicated.

of the T = 25 icosahedral capsid. Penton base subunits, each made of five molecules of the penton protein (polypeptide III), lie at the 12 vertices of the icoashedron. Projecting from the penton bases like antennae on a space satellite are fibers made of trimers of polypeptide IV. The fiber forms a triple helical structure anchored to the penton base at one end and has a terminal knob at the other end. A number of "glue" proteins (polypeptides IIIa, VI, VIII, and IX) mediate interactions between hexons and pentons, and with the viral DNA.

The 30- to 36-kb linear, double-stranded viral DNA genome is organized into coiled domains by the histone-like polypeptide VII. Basic polypeptides V and X are also bound to the viral DNA. A 55-kDa virus-coded **terminal protein** is covalently linked via a serine residue to each of the 5' ends of the viral DNA (Figure 23.3).

The viral DNA contains **inverted terminal repeat** sequences of approximately 100 bp (Figure 23.3). This means that the first 100 nt at the 5' and 3' ends of each DNA strand are complementary and can base-pair, allowing single DNA strands to circularize, forming a short panhandle end. Both the terminal protein and the inverted terminal repeats are involved in viral DNA replication (see below).

Fibers make contact with cellular receptor proteins to initiate infection

Adenoviruses attach to specific cellular receptors via the knob of the fiber protein. Most adenovirus serotypes, as well as Coxsackievirus B3 (a picornavirus; see Chapter 11) utilize the same receptor on human cells, termed *Coxsackievirus and adenovirus receptor (CAR)*. CAR is a cell surface protein of unknown function, whose amino acid sequence shows that it is a member of the **immunoglobulin family** (Figure 4.2). Rodent cells also possess a closely related CAR protein that allows their infection by human adenoviruses.

Adenoviruses types 2 and 5 also utilize certain cell surface **integrin** proteins, including integrins αVβ3 and αVβ5, as **secondary receptors**. The penton base binds to these integrins via a domain similar to that used by extracellular adhesion molecules that interact with integrins. Binding to integrins leads to the migration of virus-receptor complexes to clathrin-coated pits, where they are taken up into endosomes (see Chapter 4).

Internalization within an endosomic vesicle occurs quickly (within 10 minutes), after which the virion escapes to the cytosol in a process dependent on the acidic pH of the endosome. Virus particles are transported toward the nucleus using interactions between the hexon protein and microtubules. They localize at nuclear pores where they are disassembled in a sequential manner, and the DNA is released into the nucleus. There the viral DNA associates with the nuclear matrix via the terminal protein, and transcription of viral genes commences.

Expression of adenovirus genes is controlled at the level of transcription

Although the adenovirus gene map (Figure 23.4) seems complicated at first, when broken down into its component parts it is easier to understand. Viral genes are arranged in a well-conserved set of transcription units. Eight transcriptional promoters are used by cellular RNA polymerase II to initiate transcription, and twelve polyadenylation signals define the 3′ ends of mRNAs.

Transcription units can be divided into three classes, depending on the timing of their use during the infectious cycle. The six *early* transcription units are named E1A, E1B, E2A, E2B, E3, and E4. Note that E2A and E2B use a common promoter but different polyadenylation sites. The two *intermediate* transcription units are named IX and IVa2. Note that these genes use distinct promoters but share polyadenylation sites in common with E1B and E2B. There is only one *late* transcription unit, with a single promoter but five different polyadenylation sites. This gives rise to five families of late mRNAs, named L1 through L5.

Each of the 13 families of viral genes contains a variety of often overlapping protein-coding regions. Alternative RNA splicing generates different mRNA species that together code for a grand total of about 50 distinct adenovirus proteins. Adenovirus genomes can be seen as constructed from modular units similar to the early and late transcription units of the smaller and simpler polyomaviruses (Chapter 21). Adenovirus transcription units are interspersed in such a way that virtually all of the viral genome is utilized for coding proteins on one or the other DNA strand.

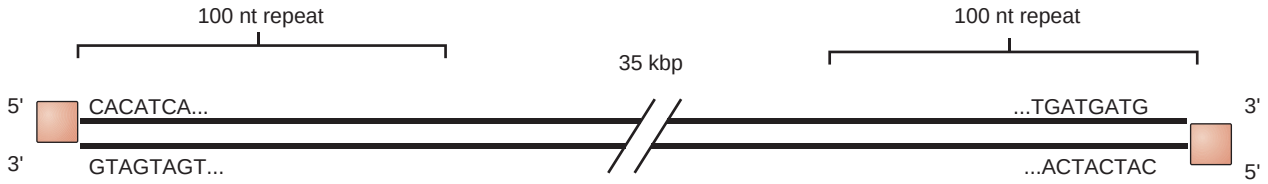

Figure 23.3 Terminal inverted repeats on adenovirus DNA. Linear, double-stranded adenovirus type 5 DNA showing the first 8 nt of the inverted terminal repeats. Terminal protein shown as an orange square.

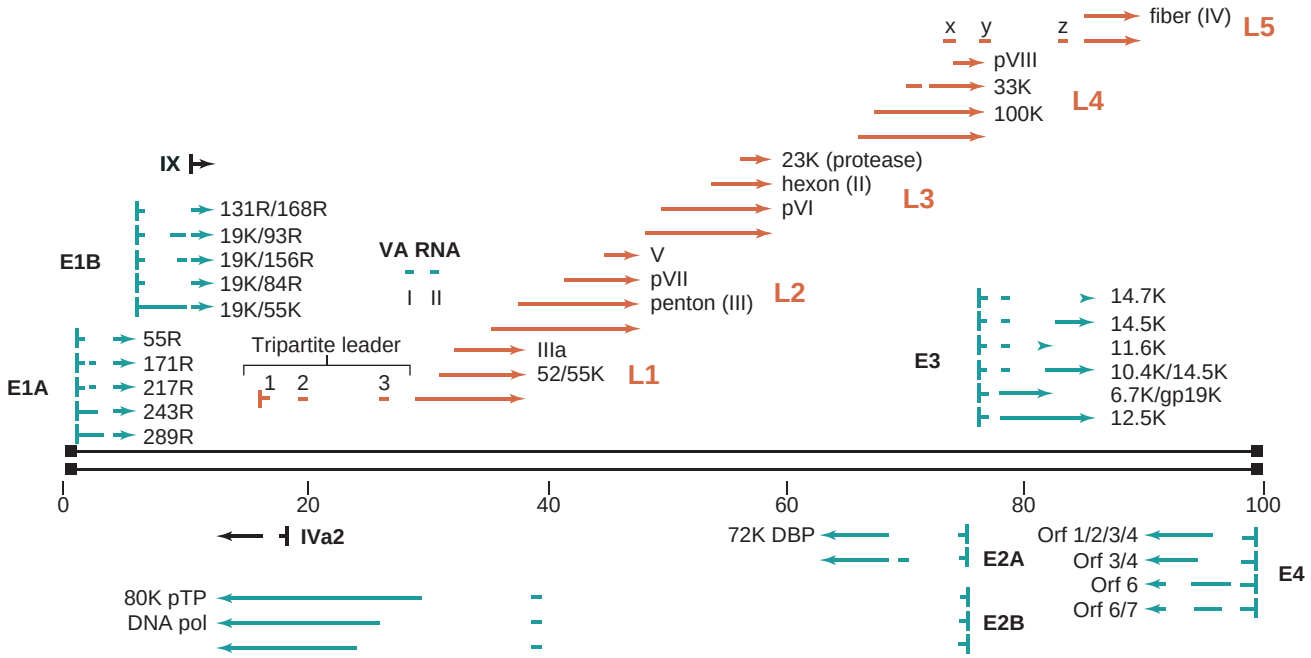

Figure 23.4 Transcriptional map and gene products of adenovirus type 5. Families of early (E, in blue), intermediate (in black), and late (L, in orange) mRNAs are shown in relation to the viral genome, divided into 100 map units (~350 nt per map unit). Rightward reading transcripts shown at top, leftward reading transcripts shown at bottom. Vertical bars show 5' ends of RNAs, corresponding to transcriptional promoters; arrowheads represent 3' ends of mRNAs, corresponding to polyadenylation sites. Introns are shown as interruptions in mRNAs. Names of proteins coded by mRNAs are shown adjacent to the corresponding mRNAs.

Adenovirus genomes also contain one or two copies of VA genes, transcribed at high levels by cellular RNA polymerase III, the enzyme used by the cell to transcribe tRNA and other small RNA genes. The small, highly base-paired VA (for "virus-associated") RNAs are involved in translational control by inhibiting activity of the cellular, double-stranded, RNA-dependent protein kinase **(PKR)**, one of the pathways by which **interferon** acts (see Chapter 33).

E1A proteins are the kingpins of the adenovirus growth cycle

E1A is the first viral gene to be transcribed. The E1A promoter, located near the left end of the genome (Figure 23.4), is driven by a transcriptional enhancer that requires only cellular transcription factors. Viruses that contain mutations in E1A genes make strongly reduced amounts of other early gene products, and therefore little DNA or progeny virions.

Two major E1A RNAs, denoted 13S and 12S (for their slightly different sedimentation rates in sucrose gradients), encode proteins of 289 and 243 amino acid residues, respectively (289R and 243R; Figure 23.5). These two proteins are identical except for a 46-amino acid region in the 289R protein that is missing in the 243R protein because of removal of a longer intron by alternative splicing. Alignment of E1A proteins from different adenovirus serotypes reveals three regions with highly conserved amino acid sequences, termed CR1 (for "conserved region 1"), CR2, and CR3 (Figure 23.5); the 243R protein is missing CR3.

The two major E1A proteins are powerful transcriptional activators of a number of viral and cellular genes. The natural target cells of adenoviruses are terminally differentiated, non-dividing epithelial cells. Adenoviruses rely on a variety of host proteins for optimal DNA replication and production of adequate levels of precursor deoxynucleotides. The E1A proteins help provide these required cellular proteins by inducing non-dividing cells to enter the S, or DNA synthesis, phase of the cell cycle. An unintended result is the ability of adenoviruses to transform non-permissive cells and to induce tumor formation in experimental animals, thus making E1A an **oncogene**.

E1A proteins bind to the retinoblastoma protein and activate E2F, a cellular transcription factor

How do E1A proteins induce cells to enter the S phase of the cell cycle? The best-understood mechanism involves an interaction between E1A proteins and the **retinoblastoma (Rb)** family of cellular tumor

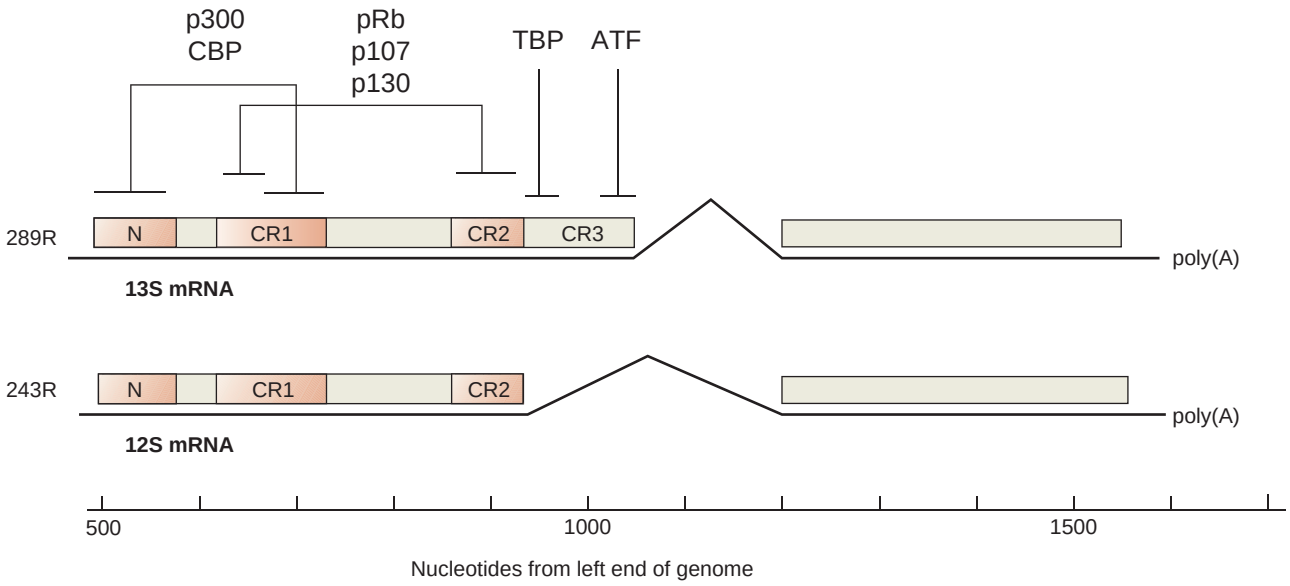

Figure 23.5 Major E1A proteins and interactions with cellular proteins. The two major E1A mRNAs are shown; introns removed by splicing are shown as inverted V's. Reading frames for the 289-amino acid and 243-amino acid protein products are shown (boxes) with the critical amino terminal region (N) and conserved regions (CR1, 2, and 3). The domains involved in binding by histone acetyl transferases (p300/CBP), Rb family members, and cellular transcription factors TATA-binding protein (TBP) and ATF are indicated.

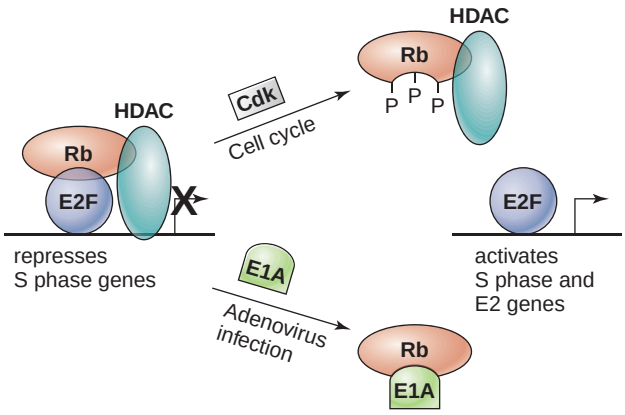

Figure 23.6 S-phase gene activation by E1A proteins. A complex between Rb and E2F binds to cellular and viral genes, and bound histone deacetylase (HDAC) represses transcription by altering chromatin structure. This complex is disrupted either during the cell cycle, by phosphorylation of Rb by cyclin-dependent kinase (Cdk; above), or during infection, by binding of adenovirus E1A proteins (below), allowing E2F to transcriptionally activate cellular and viral genes.

suppressor proteins (Figures 23.5 and 23.6). Rb was first identified as a gene that is deleted or mutated in human retinoblastomas and other cancers. Proteins of the Rb family bind to and inactivate a family of cellular transcription factors, termed E2F. E2F was first described as a transcription factor regulating the adenovirus E2 promoter (hence its name), but it also regulates promoters of many genes coding for cellular proteins involved in DNA synthesis and cell-cycle progression factors.

Rb and its family members p107 and p130 contain a *pocket domain* that binds to both the E2F and E1A proteins (Figure 23.6). **Histone deacetylases** (HDACs) bound to Rb are targeted to E2F-regulated genes and induce transcriptional repression by compressing chromatin structure, reducing access of proteins that activate transcription. In the G1 phase of normally cycling cells, Rb becomes phosphorylated by **cyclin-dependent protein kinases** (Cdks), and the phosphorylated Rb no longer binds to E2F. Thus free of repression, E2F can activate expression of S-phase genes, allowing progression into S phase.

The E1A proteins activate E2F by a similar mechanism: they bind to the Rb pocket, releasing E2F from the embrace of Rb. E2F is therefore free to activate transcription of its target genes (Figure 23.6). At the same time, E2F activates both viral genes E2A and E2B by binding to a site near their common promoter. This leads to production of three early viral proteins that direct adenovirus DNA replication.

E1A proteins also activate other cellular transcription factors

The E1A 289R protein independently activates transcription of adenovirus E3 and E4 genes by interactions between its unique CR3 domain and cellular transcription factors. CR3 contains two regions involved in transactivation: a zinc finger that interacts with the TATA-binding protein (TBP) of the basal transcription machinery, and a promoter-targeting region that activates cellular transcription factors such as ATF (Figure 23.5).

These interactions stabilize the transcription complex and stimulate viral gene expression.

Finally, the E1A amino terminus and part of CR1 engage in a complex with a distinct set of cellular proteins, the **histone acetyl transferases**. These enzymes, including p300 and the cyclic AMP-response element-binding protein, CBP (Figure 23.5), play an important role in the transcriptional activation of large blocks of cellular genes. This is carried out via the acetylation of nucleosomal histones and other proteins, resulting in a more relaxed chromatin structure ("**chromatin remodeling**") that is more accessible to specific transcription factors and is more easily transcribed. Thus these proteins reverse the transcriptional repression effects of the histone deacetylases referred to above. Binding of E1A proteins to histone acetyl transferases increases histone acetylation and thus promotes entry into the cell cycle by enhancing expression of cell-cycle genes.

E1A proteins indirectly induce apoptosis by activation of cellular p53 protein

Expression of E1A proteins leads indirectly to stabilization and activation of the cellular **tumor suppressor protein**, p53 (Figure 23.7; see also Figure 22.6). p53 is a transcription factor that both activates and represses transcription, and functions to police the natural progression of the cell cycle. Activation of p53 serves as a warning signal to the cell that something is wrong, and activated p53 induces growth arrest and cell death by **apoptosis**.

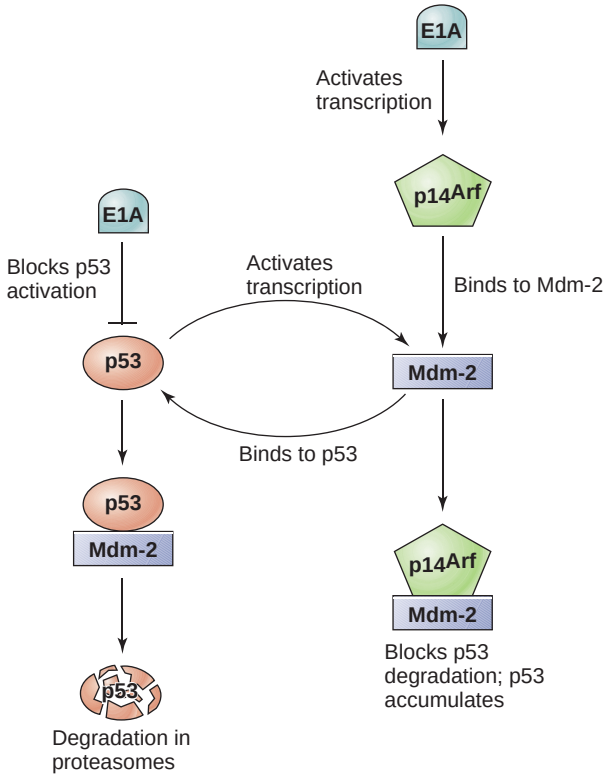

Figure 23.7 Degradation pathway of p53 is blocked by E1A proteins. See text for details.

Apoptosis is a regulated form of cell suicide invoked when cells undergo extensive DNA damage, virus infection, or other insults. p53 activity is regulated in part by at least two cellular proteins that control its stability in opposite ways. Mdm-2 binds to p53 and targets it for **ubiquitin**-mediated degradation, whereas $p14^{Arf}$ binds to Mdm-2 and blocks its binding to p53, thus stabilizing p53.

E1A proteins stabilize and activate p53 by two mechanisms (Figure 23.7). First, by binding to Rb (Figure 23.6) they activate expression of $p14^{Arf}$, which is encoded by an E2F-inducible gene. As a result, increased levels of $p14^{Arf}$ bind to Mdm-2 and reduce its level in the cell. Second, the association of E1A with p300/CBP prevents p53 from activating transcription of the Mdm-2 gene, further reducing the level of Mdm-2. The resulting accumulation of p53 could induce the early death of infected cells by apoptosis and severely limit virus production were it not reversed by other adenovirus gene products, particularly two E1B proteins.

E1B proteins suppress E1A-induced apoptosis and target key proteins for degradation, allowing virus replication to proceed

The E1B region also produces multiple mRNAs by alternative splicing. The two best characterized proteins, denoted 19K and 55K (for their molecular weights), are produced from a single 2.2-kb mRNA by initiation of translation in two different reading frames.

The 19K protein is a functional homologue of the cellular protein Bcl-2, which is a suppressor of apoptosis. Oligomers of another Bcl-2 family member, called Bax, stimulate the **caspase** cascade and lead to apoptotic cell death (see Figure 33.4). Bcl-2 reverses this effect by binding to Bax, preventing oligomer formation. Both Bcl-2 and Bax are associated with mitochondrial membranes in a complex that regulates apoptosis. The adenovirus 19K protein mimics Bcl-2 by binding to Bax and preventing oligomer formation, thereby blocking apoptosis (Table 23.1).

The 55K protein binds directly to p53 and blocks transcription from p53-dependent promoters, therefore inhibiting p53-dependent apoptosis. More importantly, 55K and a protein coded by the adenovirus E4 transcription unit, called E4orf6 (for "open reading frame 6"), form an E3 ubiquitin ligase complex, involving members of the cellular Cullin protein family and Elongins B and C, which targets bound proteins for degradation in the proteasome. p53 bound to this complex is ubiquinated, transferred to the proteasome, and degraded (Table 23.1). A growing list of other key cellular proteins are also targeted for degradation by this complex.

Table 23.1 Functions of adenovirus E1B proteins

Protein	Function	Cellular proteins involved
19K	• Suppresses apoptosis	• Binds to Bax (homologue of Bcl-2)
55K	• Suppresses apoptosis	• Binds to p53 (transcriptional repression)
	• Induces p53 turnover (with E4orf6)	• Binds to p53 and E4orf6 in complex with cellular Cullin-Elongin B/C E3 ubiquitin ligase complex
	• Promotes transport, stabilization and translation of viral mRNAs (with E4orf6)	• Requires E4orf6 and E3 ubiquitin ligase complex
	• Host cell shut-off (with E4orf6 and L4-100K)	• eIF4E, eIF2α, eIF4G

Thus the two major E1B proteins protect infected cells from both p53-dependent and p53-independent apoptosis, reversing activities stimulated by E1A proteins. This allows the cell to survive long enough to produce high levels of progeny adenovirus virions before eventually succumbing to the effects of virus infection.

The preterminal protein primes DNA synthesis carried out by viral DNA polymerase

Once E1A has induced the cell to enter S phase and the early viral gene products have accumulated, viral DNA replication begins. Three essential proteins made by E2 genes carry out viral DNA replication. A single-stranded DNA binding protein (ssDBP) is produced from the E2A region, while both a viral DNA polymerase and an 80-kDa preterminal protein (pTP) are encoded by E2B (Figure 23.4).

DNA replication can begin at either end of the linear DNA molecule (Figure 23.8). The replication origins, including the first 50 nt of each inverted terminal repeat, contain binding sites for a complex between the adenovirus DNA polymerase and preterminal protein. Binding by cellular transcription factors called nuclear factor I and nuclear factor III (also known as Oct I) enhances the efficiency of initiation of DNA replication, perhaps by increasing the affinity of the preterminal protein/DNA polymerase complex for the replication origin.

The first step in DNA replication is unusual. Instead of an RNA primer (used by cells and by most DNA viruses) or a DNA primer (used by parvoviruses), adenoviruses use the preterminal protein as a primer for replication. This protein contains a serine residue whose hydroxyl group forms a phosphodiester bond with deoxycytidine, in a reaction that cleaves dCTP to dCMP. This covalently bound dC residue is then used as a primer by the viral DNA polymerase for the further addition of nucleotides complementary to the template DNA strand (step 2, Figure 23.8).

As the DNA polymerase extends the growing DNA chain, it displaces the other strand of parental DNA, which is then coated with the single-stranded DNA binding protein. Synthesis proceeds to the end of the linear DNA, producing a double-stranded progeny DNA containing a parental and a daughter strand, and a displaced parental single-stranded DNA molecule (step 3).

Single-stranded DNA is circularized via the inverted terminal repeat

At this point the double-stranded progeny DNA can serve as a template for further replication, but in each round only one double-stranded DNA molecule is formed, and there would therefore be no net accumulation of viral DNA. To solve this problem, we come back to the inverted terminal repeat sequences on adenovirus DNA. These sequences allow the two ends of a full-length, single-stranded DNA molecule to anneal over the first 100 nt, forming a panhandle structure with a short, double-stranded stem (step 4). This stem recreates the replication origin found at both ends of the parental double-stranded DNA molecule. Therefore, the DNA polymerase-preterminal protein complex can bind and initiate DNA replication (step 5).

Once the DNA polymerase has copied the first 100 nt of the double-stranded DNA stem, it proceeds along the single-stranded DNA, displacing the single-stranded, DNA-binding protein as it goes (step 6). The final result is a second double-stranded DNA molecule (step 7, bottom of Figure 23.8). Therefore, beginning with one adenovirus DNA molecule, these two pathways together produce two double-stranded progeny molecules.

In an alternative pathway (not shown), replication can begin at both ends of a double-stranded DNA molecule, as both ends are identical. This also gives rise to two progeny genomes, as the DNA polymerase molecules pass each other on the complementary DNA strands like trains on separate tracks. There is evidence that both pathways are used. During assembly of virions the 80-kDa preterminal protein is cleaved to generate the 55-kDa terminal protein, which remains covalently attached to the genome after packaging.

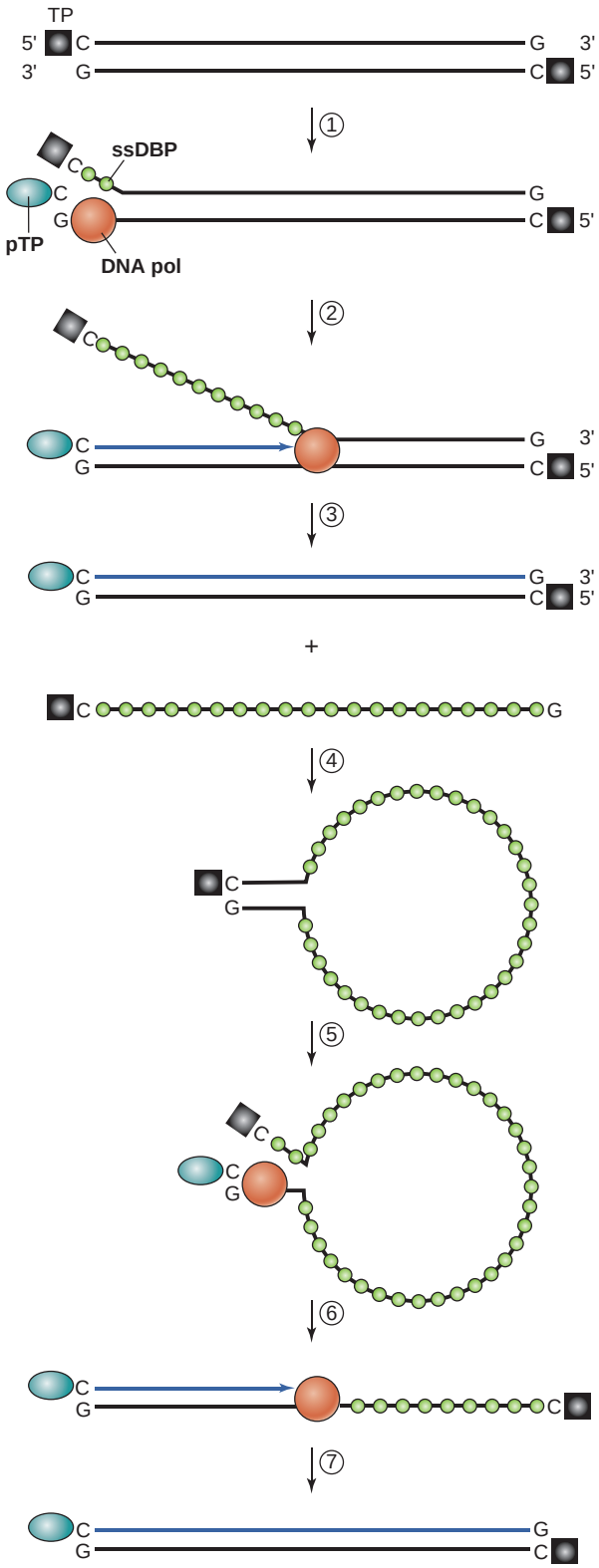

Figure 23.8 Adenovirus DNA replication. Viral DNA is replicated by three viral proteins: preterminal protein (pTP, blue oval), DNA polymerase (DNA pol, orange circle), and single-stranded DNA binding protein (ssDBP, small green circles). The cleaved, 55 kDa terminal protein (TP) is represented by a black square. Newly synthesized DNA is shown in blue. See text for details.

The major late promoter is activated after DNA replication begins

More than half of the adenovirus genome is dedicated to coding for at least 18 different late mRNAs. These mRNAs encode structural proteins, scaffolding proteins, a protease, and other products required for packaging of progeny virus. All late mRNAs are produced from a single primary transcript that extends 29 kb in the rightward direction from the highly active "major late promoter", at 16.5 map units (Figures 23.4 and 23.9). During the early phase this promoter has a low activity, and the rare transcripts terminate prematurely, so that only low levels of L1 mRNAs are made. Transcription of late genes is activated after the onset of viral DNA replication, and late transcripts become the major RNAs made in the infected cell.

At least two factors control the increase in late transcription. First, newly replicated viral DNA interacts with a different set of DNA binding proteins, thus altering DNA structure to make the major late promoter more accessible to cellular transcription factors. Second, polypeptide IVa2, which is encoded by an intermediate gene, binds to viral DNA at a site shortly downstream of the position where late transcription begins. IVa2 cooperates with factors bound to an upstream enhancer to activate transcription. Together, these steps activate the major late promoter by a factor of 100 or more.

Five different poly(A) sites and alternative splicing generate multiple late mRNAs

There are five families of late mRNAs, denoted L1 to L5, each defined by a unique polyadenylation site (Figures 23.4 and 23.9). Each primary transcript from the major late promoter is cleaved at only one of these sites, and the resulting polyadenylated RNA is spliced in a complex fashion to generate one of the 18 possible late mRNAs. Three short exons in the first 4 kb of these transcripts are spliced together to form the noncoding 200-nt "tripartite leader". This leader is then spliced to an mRNA "body"; multiple alternative 3' splice sites give rise to several different mRNAs for each of the five late gene families. As a result, all late mRNAs have the same tripartite leader at their 5' ends. Each of these mRNAs is translated into a unique protein by use of reading frames beginning at different AUG initiation codons within the mRNA body.

The tripartite leader ensures efficient transport of late mRNAs to the cytoplasm

What are the advantages to adenovirus in expressing its late mRNAs in such a fashion? First, all late mRNAs are made by turning on a single, highly active promoter, allowing coordinate expression of late proteins when

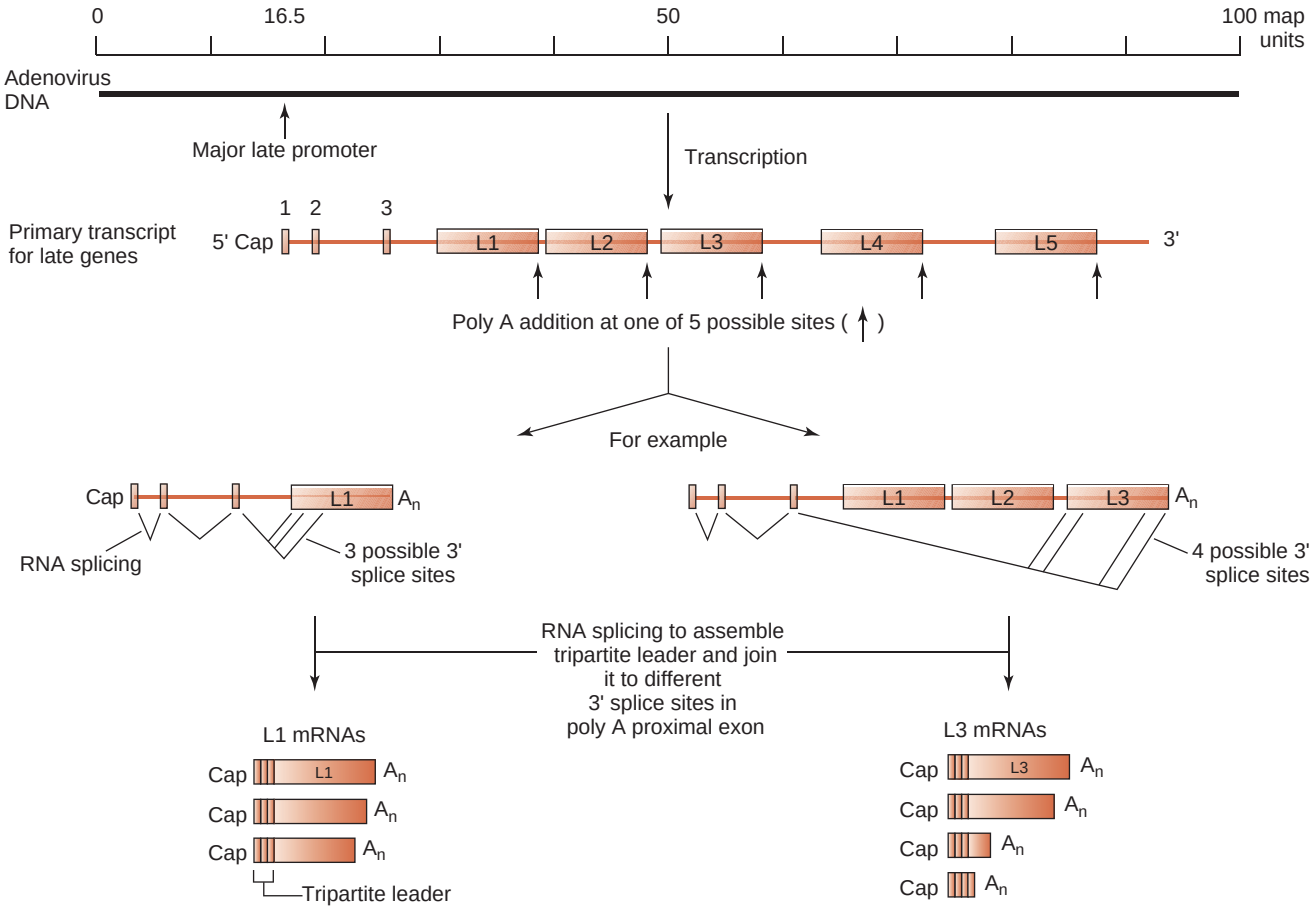

Figure 23.9 Production of late adenovirus mRNAs. Transcription initiates at the major late promoter, and the RNA is cleaved and polyadenylated at one of 5 possible sites. The tripartite leader is formed by splicing 3 small exons; it is spliced to the mRNA body at one of several alternative 3' splice sites. Exons are shown as shaded rectangles.

they are needed, after DNA synthesis begins. Second, all late mRNAs contain the same leader sequence at their 5' ends. This allows the mRNA transport and translation machinery to concentrate on these RNAs.

The Cullin-based E3 ubiquitin ligase complex discussed above, containing the E1B 55K protein and the E4orf6 protein, is responsible for selective transport of late mRNAs to the cytoplasm (Table 23.1). E4orf6 targets the 55K protein to the nucleus, but both 55K and E4orf6 also contain nuclear export signals that are involved in shuttling these complexes out of the nucleus. It is still uncertain whether direct shuttling, targeted protein degradation, or both are responsible for the selective transport and stabilization of viral mRNAs. Nevertheless, the effect is that late adenovirus mRNAs predominate in the cytoplasm during the late phase of infection.

The tripartite leader directs efficient translation of late adenovirus proteins

The cellular translation initiation factor eIF4F normally binds to the 5' cap structure on mRNAs and enables the 40S ribosomal subunit to scan mRNAs for an appropriate AUG initiation codon (see Figure 11.5a). EIF4F is dephosphorylated by an adenovirus host shutoff protein during the late phase of infection, and it can no longer direct scanning of mRNAs, resulting in the inhibition of translation of most cellular mRNAs. However, viral mRNAs containing the tripartite leader continue to be translated efficiently, because the leader allows **ribosome shunting** that avoids the scanning mechanism. Shunting is directed by complementary regions between the tripartite leader and the 18S RNA, which is part of the 40S ribosomal subunit. Ribosome shunting has parallels to initiation of protein synthesis at "internal ribosome entry sites" (IRES elements) of picornaviruses and flaviviruses (see Chapters 11 and 12).

Adenoviruses make different amounts of the various late mRNAs (and therefore of their protein products) by controlling which polyadenylation sites and 3' splice sites are recognized most efficiently. This system, using a single transcription unit and producing multiple mRNAs by different RNA processing reactions, may be a simpler and more economical means than having

distinct transcriptional enhancers and promoters for each of the many late mRNAs needed.

Adenovirus-induced cell killing

Two viral proteins have been implicated in cell killing and virus release. One is the E3 11.6-kDa "death protein". Although it is nominally an early protein, the 11.6-kDa protein is expressed at high levels during the late phase when its mRNA is made using the major late promoter and an E3 polyadenylation site. The death protein is a membrane glycoprotein found in the Golgi membranes, endoplasmic reticulum, and most predominantly in the nuclear membrane; how it kills cells is not known.

The E4orf4 protein also can induce cell death, via a p53-independent mechanism. When expressed alone, E4orf4 kills a wide range of cancer cells, but it has little effect on normal human cells. This specificity of killing suggests that E4orf4 could be of use therapeutically in the treatment of human malignancies (see below). Although it has not been proven to be involved in the death of adenovirus-infected cells, E4orf4 could play an important role.

Cell transformation and oncogenesis by human adenoviruses

Adenoviruses (and other DNA tumor viruses) can oncogenically transform cells as a result of their ability to induce active cycling in cells that are normally in a resting state. Transformed cells are not subjected to normal control of the cell cycle, and therefore grow and divide in an unregulated fashion.

Adenovirus infection of human cells usually results in cell death, and therefore no transformed cells can survive. The human cell line 293 was generated *in vitro* by transfection of fetal embryonic kidney cells with a fragment of adenovirus DNA containing only the E1A and E1B genes, allowing survival and growth of transformed cells. Although rodent cells can be readily infected by human adenoviruses, they are non-permissive for viral replication and therefore survive infection. Virtually all human adenoviruses are capable of transforming rodent cells in culture, and such cells usually form tumors when injected into newborn or immunologically compromised rodents. However, only group A adenoviruses yield tumors by direct injection of virus into animals.

Therapeutic applications of human adenoviruses

In the near future we will almost certainly see adenoviruses used in a variety of clinical applications. Their ability to infect a wide range of cells and to express genes at high levels make them ideal tools to fight disease in humans and other mammals. Adenoviruses can be readily manipulated to allow expression of almost any gene by insertion of the gene under control of a suitable promoter into the viral genome.

Vaccines and gene therapy. As discussed in Chapter 37, human adenoviruses are already being used experimentally in gene therapy. In this application they function primarily as vectors to deliver genes that correct genetic deficiencies associated with human diseases. Most of the commonly used adenovirus vectors are missing E1A and E1B genes (and often many more) and therefore cannot replicate in normal cells. Replication-competent adenovirus vectors could also be used as vaccines to enhance the immune response against any desired target. Numerous genes have been inserted into adenovirus vectors for experimental vaccination, including genes encoding hepatitis B surface and core antigens, herpes simplex glycoprotein, rabies virus glycoprotein, respiratory syncytial virus F protein, and several HIV-1 proteins.

New cancer therapies. In a novel approach currently being tested in clinical trials, a replication-competent adenovirus mutant is injected into human tumors. The idea is that the virus will replicate in and spread through the tumor tissue, killing the cancerous cells. The virus strain, ONYX-015, is a mutant that does not express the E1B 55K protein, which plays a vital role in inactivating and degrading p53. It was hypothesized that in normal cells infected with this virus p53 will remain active, leading to poor replication of the virus, little spread, and only limited pathological consequences. However, more than 50% of human tumors lack p53; in such cells, inactivation of p53 by the 55K protein is not required for virus replication, and therefore the ONYX-015 virus replicates actively in tumor cells and kills them selectively. While ONYX-015 does seem to kill cancer cells selectively, the basis for this effect now seems more complex than simply the presence or absence of p53. This virus is still being tested in clinical trials.

Clinical use of other adenovirus functions. Viral proteins have evolved to modulate many aspects of cell growth, apoptosis, and the immune system. It should be possible to take advantage of these viral proteins to develop new therapies. Such proteins could be expressed in target cells using viral vectors. In addition, understanding their mechanism of action could identify new targets for the development of drugs that mimic the action of the viral protein. Both E1B 19K and 55K proteins are potent inhibitors of apoptosis, and could be of value in diseases characterized by excess or unwanted apoptotic cell death, such as certain neurological disorders (e.g., Alzheimer's disease) or stroke. The E4orf4 protein kills cancer cells selectively and could be applied to the treatment of human malignancies. Finally, several E3 proteins reduce the immune response, and could be used in immune suppression therapy. Disease-causing viruses such as human adenoviruses may soon be developed into valuable new therapeutic agents.

In human adenoviruses, E1A is the oncogene that stimulates uncontrolled cell-cycle progression. Expression of E1A alone in rodent cells usually produces abortive transformation. In this process, transformed cells begin to proliferate, but die soon afterward due to E1A-induced apoptosis. Formation of stable transformants requires coexpression of E1A and either E1B 19K or E1B 55K proteins, preferably both. As discussed previously, these E1B proteins blunt the pro-apoptotic effects of E1A, and inhibit p53-induced growth arrest and apoptosis. This system has provided valuable tools to understand the more general roles of the Rb and p300/CBP families, cell-cycle progression genes, and p53.

KEY TERMS

Adenoid
Apoptosis
Caspase
Chromatin remodeling
Cyclin-dependent protein kinase
Hexon
Histone acetyl transferase
Histone deacetylases (HDACs)
Immunoglobulin gene superfamily
Integrins
Interferon
Inverted terminal repeat
Oncogene
Penton base
PKR
Retinoblastoma (Rb)
Ribosome shunting
Secondary receptor
Terminal protein
Tumor suppressor protein
Ubiquitin

FUNDAMENTAL CONCEPTS

- Many human adenoviruses bind to both a primary receptor (CAR) and a secondary receptor (integrin), which leads to uptake in endocytic vesicles.
- Adenovirus genes are organized in six early, two intermediate, and five late families.
- E1A proteins bind to cellular Rb protein, dissociating it from cellular E2F protein and stimulating transcription of other viral genes and numerous cellular genes that bring non-dividing cells into the cell cycle.
- E1A proteins also engage cellular histone acetyl transferases, resulting in relaxed chromatin and increased transcription of many cellular genes.
- E1A proteins indirectly induce apoptosis by increasing levels of cellular p53 protein.
- E1B proteins reverse E1A-induced apoptosis by reducing p53 levels.
- Viral DNA synthesis begins at either end of the linear genome with a protein primer covalently bound to a nucleotide.
- Eighteen different late viral mRNAs are made from a single transcription unit by use of five polyadenylation signals and multiple splicing sites.
- Some adenoviruses can induce cancer in experimental animals but not in humans.

REVIEW QUESTIONS

1. Discuss the principal methods used by adenoviruses to generate approximately 50 distinct adenovirus proteins by using a very limited number of transcriptional promoters.

2. How do the adenovirus E1A proteins induce apoptosis via p53?

3. DNA replication initiating on a single-stranded DNA template requires a primer. What is the primer for initiating adenovirus DNA replication?

4. Adenovirus inhibits a cellular factor that binds to the 5' cap structure on mRNA and enables the 40S ribosomal subunit to begin scanning the mRNA for an appropriate AUG initiation codon. Nevertheless, late adenovirus mRNAs are efficiently translated; by what mechanism?